Editorial

Vascular Remodeling
Honey, I Think I Shrunk the Artery

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The mechanisms responsible for both favorable and unfavorable outcomes of balloon angioplasty continue to invite controversy. Nowhere is this more profound than in the case of restenosis, clearly the most frequent complication of percutaneous revascularization. Despite the fact that 185 patents describing treatment strategies designed to limit restenosis have been issued during the past decade,1 until recently successful clinical application of derivative therapies has been virtually without success. There can be little doubt that our lack of understanding regarding the mechanisms responsible for restenosis has provided the underpinnings of our inability to successfully prevent its recurrence.

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The vast majority of medical therapies designed to preempt restenosis have been predicated on the assumption that smooth muscle cell (SMC) proliferation constitutes the principal pathogenetic basis for restenosis. This concept has its origins in the fundamental studies of human atherosclerotic arteries that identified SMC accumulation within the neointima of (primary) lesions obtained at necropsy2-4 and in support of experimental observations in a variety of animal models.5-10 Subsequently, beginning with the report of Austin et al,11 necropsy examination of sporadic patients dying at various intervals after percutaneous revascularization12-21 disclosed foci of hypercellularity, including cells with phenotypic characteristics of vascular SMCs, at the original site of balloon angioplasty.

These reports were subsequently amplified by systematic examination of larger numbers of patients in whom percutaneous revascularization was accomplished by directional atherectomy. In particular, studies by Johnson et al,22 Safian et al,23 Garratt et al,24 and Strauss et al25 suggested that the histological findings observed at necropsy could be used to distinguish primary from restenotic lesions. Primary lesions appeared typically hypocellular, consisting predominantly of well-organized collagen and ambiguous ground substance. In contrast, restenotic lesions typically included a focus of hypercellularity; as observed at necropsy, cells within these foci typically had phenotypic characteristics of proliferative vascular SMCs, and the matrix surrounding these cells typically had a distinctly lighter hue and less-compact appearance than the matrix of primary or adjacent plaque.26

These contrasting findings regarding primary and restenotic lesions were perhaps best illustrated in a group of 18 patients studied in our laboratory27 in whom directional atherectomy had been performed both as the primary intervention and again when the patient returned with restenosis. These 18 patients thus offered a unique opportunity to study the same lesion site in the same artery of the same patient at two different points in time. Light microscopic examination documented distinctive features, including hypercellular foci consisting of proliferative vascular SMCs surrounded by a loose neomatrix in 13 of 18 cases (72%). In 5 important exceptions, however, neither the primary nor the restenotic specimen demonstrated such a “restenosis focus.” Of 253 restenosis specimens (65%) retrieved by directional atherectomy and studied in our laboratory,27 a similar restenosis focus was identified in 165 (65%); among the remaining 88 specimens (35%), however, no distinctive histological features were observed.

Additional evidence supporting the proliferative nature of restenosis versus primary lesions is derived from in vivo studies of SMCs cultured directly from explanted fragments of human atherectomy specimens.28-30 These studies documented that the outgrowth kinetics of SMCs cultivated in this manner were indeed a reflection of the lesion type. Among 41 lesions retrieved by directional atherectomy and used in our laboratory to initiate cell growth,29 the mean proportion of explant fragments yielding outgrowth, per lesion, was 69±4%. This varied according to the nature of the lesion. Specifically, the prevalence of outgrowth from the restenotic lesions (81±3%) was greater than that obtained from the primary lesions (56±6%, P<.001).

The time course of initiation of SMC outgrowth also varied according to the nature of the lesion. Initiation of outgrowth occurred most rapidly among explants derived from restenotic lesions and was half-maximal by 5.9±0.6 days. This was significantly earlier than that of the primary lesions (8.7±0.4 days, P<.001). The initiation of outgrowth was clearly more immediate in the restenotic lesions. By 5 days, SMC outgrowth had begun among 32±4% of the explants from restenotic lesions versus 9±5% of explants from primary lesions (P<.001). Statistically significant differences between the groups persisted throughout the outgrowth period. The rate of accumulation of cells around the explant was also substantially higher in tissue from restenotic

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lesions than that from primary plaque. Twenty-one days after the half-maximal outgrowth was attained, the total number of cells per adherent explant was 2791 ± 631 for restenotic tissue and 653 ± 144 for primary tissue (P < .01).

Most recently, the contribution of SMC proliferation to restenosis has been analyzed by examining certain cell cycle markers of cellular proliferation, such as proliferating cell nuclear antigen (PCNA), in both primary and restenotic atherosclerotic lesions retrieved by directional atherectomy. PCNA is an essential cofactor for DNA polymerase δ,31,32 and its presence in the cell is considered to be a specific indication that the cell is replicating.33 Gordon and coworkers34 had considered previously that PCNA immunostaining to quantify proliferation in the coronary arteries of hearts explanted from patients undergoing cardiac transplantation. In that patient population, some atherosclerotic lesions (3 of 14) displayed no evidence of proliferation, and the overall proportion of PCNA-positive cells was relatively low (mean, 0.85%).

In contrast, immunohistochemical analyses applied in our laboratory to atherectomy specimens retrieved from actively symptomatic patients disclosed that 5 of 7 primary and 8 of 8 restenotic lesions contained proliferating cells.35 The proliferative index (percent of PCNA-positive cells) was higher in restenotic (15.2 ± 13.6%) than in primary (3.6 ± 3.5%) lesions. Proliferating cells were detected as late as 1 year after angioplasty. To verify these observations, we studied a parallel series of 22 additional lesions, using, as a wholly independent technique, in situ hybridization. Among the 22 plaques analyzed by in situ hybridization, 7 of 11 primary and 11 of 11 restenotic lesions contained PCNA-positive cells. The proliferative index assessed by in situ hybridization was 7.2 ± 10.8% in primary lesions and 20.6 ± 18.2% in restenotic lesions (P < .05). If one assumes that the generation time5 of SMCs is similar for primary and restenotic lesions, then these findings suggest a higher rate of SMC proliferation among the restenotic lesions.

As indicated above, not all atherectomy specimens retrieved from restenotic lesions demonstrate foci of hypercellularity. This is particularly true of restenotic coronary—as opposed to lower extremity—lesions. Among the specimens analyzed for PCNA in our laboratory, 25 of 37 (67%) were retrieved from the lower extremity vasculature. O’Brien et al27 studied exclusively coronary atherectomy specimens and found a much lower incidence of specimens with PCNA-positive cells. Some of the discrepancy between our observations and the findings reported by O’Brien et al may be attributable to methodological issues: all of the specimens analyzed by immunohistochemistry in our laboratory were preserved overnight in methanol to minimize the loss of antigenicity. Furthermore, no attempt was made in our laboratory to index PCNA staining to an S-phase specific marker such as thymidine or thymidine analogues such as bromodeoxyuridine; because PCNA expression is not limited to S-phase, but occurs during G1 and G2 as well, only a fraction of those cells labeled by PCNA will be in S-phase, and any attempt to limit counts to those corresponding to S-phase will predictably result in a lower index of cell proliferation. Aside from these methodological issues, however, it is entirely possible that some of the reported variance with regard to the issue of cell proliferation relates to the size of the artery studied. The extent of cellular proliferation required to renarrow a larger (eg, peripheral) artery may exceed that required to renarrow a smaller (eg, coronary) artery; thus, the likelihood of retrieving a piece of tissue with evidence of ongoing cellular proliferation may be reduced in smaller arteries.

Even if one could obviate all such methodological concerns, however, there is little doubt that SMC proliferation alone cannot account for all restenosis events or even the full extent of lesion recurrence in any individual case. The evidence for this has been considered previously38,39 and has resulted in a number of restenosis “anti-theories” indicating periprocedural thrombosis, recoil, and plaque hemorrhage as possible alternative pathogenetic mechanisms. The controversy is by no means academic for if one accepts that “restenosis” embraces a variety of disordered responses to percutaneous revascularization, then the clear implication is that a variety of therapeutic responses may be required to achieve sustained luminal patency. Indeed, the repeated failure of multiple clinical trials to demonstrate efficacy of a given intervention may have resulted in part from the unjustified expectation that interventions designed to defeat one specific mechanism could not be sabotaged by alternative mechanisms. Even when preliminary reports suggest a reduction in restenosis frequency as the result of endovascular stents40,41 or platelet-receptor antagonists,42 the treatment failures that persist in such trials may again represent alternative mechanisms requiring alternative therapies.

In the current issue of Circulation, Post et al43 propose yet another potential pathogenetic mechanism, which they have called “remodeling,” to explain those cases of restenosis that lack a significant proliferative component. While no attempt was made to directly investigate cellular proliferation in the various animal models used, the authors observed a consistent deficit between the magnitude of late lumen loss measured by serial quantitative angiography and the diameter reduction that could be attributed to histologically identifiable intimal thickening. They concluded that “the deficit appeared to be due predominantly to reduction in the area circumscribed by the internal elastic membrane.” Because the internal elastic membrane demarcates the native arterial lumen, any reduction in lumen area unassociated with plaque growth must, by default, be due to “constriction” of the lumen—the term used by others44 to describe similar findings—by a “shrinking” arterial wall. Post et al estimated that remodeling accounted for 52% to 89% of the late loss in their in vivo experiments. Conversely, neointimal proliferation appeared to be responsible for an insignificant fraction of the late lumen loss in most cases.

As Post et al acknowledge, the models and methods that they have used in their seminal attempts to discern the contribution of remodeling to restenosis have certain important limitations. First, in four of the five animal models that they used, balloon angioplasty was applied to structurally normal arteries without adjunctive dietary manipulations typically required to generate robust neointimal lesions. Most animal models that have been used to study the impact of mechanical or
pharmacological therapies on restenosis have included administration of dietary supplements in conjunction with balloon injury of the arterial wall for the simple reason that balloon injury applied as a solitary provocation to the peripheral arteries of rabbits or swine rarely results in significant luminal narrowing. Consequently, it is not surprising that intimal thickening contributed little to whatever reduction in luminal patency developed over the ensuing 3 to 8 weeks. In the case of normal arteries subjected to moderate inflation pressures (5 bar), balloon trauma appears to have been limited to a combination of stretching and an indeterminate amount of endothelial cell denudation; correspondingly, the magnitude of late loss calculated for initially normal arteries appears to comprise the combined loss of initial gain achieved by stretching (i.e., graduated recoil), a small amount of neointimal thickening, and the remaining “deficit” attributed by the authors to remodeling.

The second methodological issue concerns the fact that among all of the animal models described in these studies—including the “atherogenic” swine—the severity of luminal narrowing achieved with either the use of standard balloon inflation or inflation of a radiofrequency-heated balloon was not quantified and limited to only “mild” disease. When arteries that are only “mildly” narrowed by atherosclerotic plaque are prepared for histological analysis, the process of alcohol dehydration, solvent clearing, and paraffin embedding typically leads to a marked reduction in total arterial cross-sectional area but does not alter the area of the arterial wall (including neointima) itself; as a consequence of these changes, cross-sectional area of the arterial lumen is spuriously reduced, thus overestimating percent luminal narrowing.45,46 Thus, the apparent deficit attributed by Post et al to remodeling in these mildly narrowed arteries could just as easily represent an artifact of tissue processing.

Third, and perhaps the issue of greatest concern, is the choice of methods selected to identify the putative deficit that constitutes remodeling. For in vivo studies, angiography was used to serially quantify reduction in luminal patency after angioplasty. This is an incongruous choice for a group of investigators who were among the first to recognize the value of intravascular ultrasound (IVUS) for studies of this type, particularly considering the goals of the present study. Angiography provides no information—quantitative or qualitative—regarding the arterial wall, and furthermore, it can only be used to indirectly calculate—rather than directly measure—luminal cross-sectional area.49 In the case of irregular luminal geometry typically seen after balloon angioplasty,50,51 no algorithms accurately define minimal luminal diameter. Even when luminal cross-sectional area can be directly planimetered, eg, by IVUS or at necropsy, use of luminal diameter, measured or calculated, may be misleading; this is particularly so for eccentric stenoses that constitute the bulk of coronary lesions. While it is unlikely that such eccentric morphology consistently complicated the histological analyses in the study of Post et al, this methodological issue is compounded by the absence of any direct area measurements of plaque or arterial wall.

Fourth, and more of a conceptual than practical issue, the arguments advanced by Post et al altogether omit any consideration of the classic notion of vascular remodeling, compensatory enlargement. For arteries in which intimal thickening was “mild,” compensatory enlargement of the arterial wall would have been anticipated to preserve native luminal dimensions. This observation, made initially in vitro by Glagov et al52 and more recently validated in human peripheral arteries in vivo53 cannot be so readily dismissed.

As is frequently the case with seminal observations, however, the instincts and insights of the investigators may ultimately prevail despite methodological difficulties that complicate the initial analysis. This may well apply to the concept of vascular remodeling. A remarkable proliferation of confirmatory studies54–60 suggests that vascular remodeling is indeed a real entity. Because these confirmatory studies have thus far been reported only in preliminary form, full judgment of the manner in which they refine or modify remodeling as conceptualized by Post et al must be deferred. Some of these studies, however, have addressed the methodological concerns outlined above and therefore are particularly noteworthy.

Lafont et al,64 for example, while measuring luminal diameter reduction in vivo by angiography, reported direct area measurements of intimal thickening as determined by planimetry of histological cross sections at necropsy. Comparison of their necropsy and angiographic findings confirmed the observations of Post et al.

Mintz and cowokers55–57 used IVUS to serially evaluate the acute and chronic response to percutaneous revascularization in human patients; these findings thus concern arteries narrowed to a critical degree by atherosclerotic plaque and are of course unencumbered by artifactual shrinking due to fixation and processing. Moreover, because their approach permitted direct area measurements of both wall and lumen, the authors’ findings support their contentions that late lumen loss is not limited to encroachment by atherosclerotic plaque but rather is in part related to a demonstrable reduction in total arterial cross-sectional area.

To their credit, the Dutch group has likewise used serial IVUS analyses of human patients undergoing percutaneous lower extremity revascularization61 to confirm their interpretation of their live animal studies.

The variety of methods and analyses used in these preliminary reports strongly suggests that the notion of vascular remodeling is conceptually valid. What remains to be determined, however, is the extent to and mechanism by which plastic reformation of the arterial wall accounts for restenosis, independent of neointimal thickening. As Post et al have emphasized, their study was not designed to investigate the mechanism of remodeling but simply to document its contribution to luminal narrowing after balloon injury. Nevertheless, some of their own observations in concert with those made by others are relevant to the mechanism responsible for remodeling.

In particular, the medial layer of the arterial wall is not likely to constitute the locus responsible for remodeling. In the mildly narrowed, lower extremity arteries studied by Post et al, no difference was observed in the area of the media of the arterial wall at the angioplasty site among any of the experimental groups compared with controls. Previous analysis of severely narrowed coronary arteries58 has disclosed correspondingly severe
depletion of the medial component of the arterial wall; already so emaciated, further meaningful reduction in medial thickness sufficient to account for significant arterial shrinking seems doubtful.

Adventitial fibrosis, however, may well serve as a contracting scar, behaving like a noose to constrict luminal area after revascularization. Preliminary findings supporting this concept have been recently reported by Brott et al.64 Adventitial fibrosis has been noted previously in association with progressive luminal narrowing by atherosclerotic plaque.65 Moreover, the often-overlooked observation that adventitial injury alone can lead to intimal thickening constitutes evidence for dynamic interaction between the inside and outside of the vessel wall.64,65 Recent experimental findings imply significant potential for transmural distribution of a variety of molecular species applied exclusively to the adventitia66,67; in arteries narrowed by atherosclerotic plaque, the vasa vasorum68 presumably further facilitate such transmural exchange from intima to adventitia of cells and cytokines that may modulate adventitial fibrosis as well as intimal thickening.

Post et al have thus established a novel direction for investigations of restenosis. The precise therapeutic implications of their observations, however, will depend to a great extent on whether the basis for remodeling can be shown to be discrete from that responsible for neointimal thickening. Can the former develop independent of the latter? Can it develop to the point, as suggested by both Post et al and Mintz et al,57 where remodeling constitutes the dominant mechanism of restenosis in certain patients? If so, it would be inappropriate to limit strategies designed to reduce restenosis to those that are designed to inhibit SMC proliferation. Endovascular stents were at one time viewed as a means of facilitating orderly neointimal healing.69 Do those patients in whom endovascular stents have now been shown to prevent restenosis41,42 instead represent a specific patient subset in whom arterial shrinking is precluded by placement of a mechanical scaffold? In those cases in which stents obviate remodeling but fail to prevent restenosis, what is it that constitutes the persistent stimulus for neointimal thickening within the stent? And what is the role, if any, of remodeling in the progressive reduction of native luminal patency by (primary) atherosclerosis? If past experience with the vascular biology of restenosis is any indication, our continuing attempts to understand and solve this enigmatic entity may once again teach us something new about the vascular biology of atherosclerosis itself.

References


Vascular remodeling. Honey, I think I shrunk the artery.
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